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REMARKS

Applicants submit this Amendment in response to the Office Action of April 30, 2004. The claims have been amended as follows.

Claims 24 and 25 have been amended to call for an aqueous fluid. Support for the amendment of these claims is found throughout the specification. Claim 47 has been amended to supply the missing word "is". Claims 82, 83, 84, and 86 have been amended. Support for the amendment of these claims is found in the specification in Example 3 on page 13. Claim 89 has been amended and claim 91 has been added. Support for this amendment and this new claim is also found in the specification in Example 3 on page 13.

REJECTIONS OF THE CLAIMS

I. Rejection under 35 U.S.C. §102(b)

The Examiner has rejected claims 82, 85, and 87 as being anticipated by Kata, Acta Pharm. Hung, 54:116-122 (1984). Applicants traverse the rejection of these claims on this ground.

Independent claim 82 has been amended to call for niacinamide. Claims 85 and 87 are dependent from claim 82. Kata does not disclose or suggest niacinamide.

Applicants submit that the amendment to claim 82 overcomes the basis of rejection of these claims as anticipated by the disclosure of Kata and the Examiner is requested to withdraw the rejection of these claims on this ground.

II. Rejections under 35 U.S.C. §103

A. Kata, Acta Pharm. Hung, 54:116-122 (1984), and Chien, U.S. Patent No. 4,032,645

The Examiner has rejected claims 24, 27-29, 31, 32, 40, 41, 43-58, 63, 65-68, 71-74, 76-87, and 90 as being obvious over Kata and Chien. Applicants traverse the rejection of these claims on this ground.

Preliminarily, Applicants submit that the Examiner's statement concerning the method of claims 74 and 76-79 is incorrect. The method is not merely a method for using less BCD to solubilize MTZ than would ordinarily be necessary by adding another solubilizing agent. The method of the invention provides the ability to solubilize more MTZ in aqueous fluid than would ordinarily be possible due to the limited aqueous solubility of BCD. Thus, the method of the invention permits the solubilization of higher concentrations of MTZ than would otherwise be possible even when using the maximum possible dissolved concentrations of BCD.

Kata's disclosure is rather complex and involves both studies concerning solubility of betacyclodextrin and metronidazole and dissolution (rate of dissolving) of metronidazole. On page 1, third paragraph following "Introduction", Kata discloses that metronidazole "dissolves in water at a rate of about 0.5-1%." This disclosure is consistent with the results of a metronidazole solubility study disclosed by the present inventors in U.S. Patent No. 6,468,989, at Example 1, column 6, lines 24-51, in which the maximum dissolved concentration of metronidazole in aqueous gel without the presence of a solubility enhancing agent was found to be 0.7% w/w.

In Section 2.1 and in Figure 1 of Kata at page 2, Kata discloses that metronidazole solubility increases with increasing concentration of betacyclodextrin, the data of which is shown graphically in Figure 1. The graph of Figure 1 appears to show an aqueous metronidazole dissolved concentration approaching 2% when the metronidazole was dissolved from a combination of a 60/40 ratio of betacyclodextrin to metronidazole at a temperature of 37°C.

Kata further discloses, in Section 2.3 and 3, the results of dissolution studies of various combinations of betacyclodextrin and metronidazole in 900 ml of water at 37°C. The results show that, in a proportion of 60% betacyclodextrin and 40% metronidazole, a measured quantity of such mixtures dissolves in less than 15 minutes. See Figures 2, 3, 4, and 5 on pages 4, 5, 6, and 8, respectively.

On page 11, Kata discloses the following, which was cited in part by the Examiner:

> On the basis of the foregoing we prepared aqueous solutions of 1.5% metronidazole and 2.25% β-CD; ampoules were filled with the solutions and sterilized in a Certoklav autoclave at 120°C for 20 minutes, then stored at room temperature or in a refrigerator (6°C). With a high-precision pH meter we determined that the solution was slightly acidic to neutral (pH between 5.5 and 7.3). The color of the solutions (by spectrophotometry) and the active ingredient content (also measured spectrophotometrically) are independent whether they have been sterilized or stored in a refrigerator and did not change (±3.3%).

Applicants respectfully submit that the above disclosure of Kata is incredible, in several respects, and that one skilled in the art would be unable to duplicate the results disclosed to be obtained by Kata and would understand that Kata's data is impossible. Applicants submit herewith the following:

(1) Redenti, et al., Journal of Pharmaceutical Sciences, 89(1):1-8 (2000),

(2) "Cyclodextrins", Cerestar USA, Inc., and

(3) "Modified Cyclodextrins", by Cerestar Inc.

Redenti discloses, at page 2, column 1, lines 7-12, that betacyclodextrin has limited aqueous solubility - 1.85% at 25°C, which hinders its successful application as a solubilizing agent. The "Cyclodextrins" publication by Cerestar discloses on the third page in the table entitled "Solubility in Water", that the solubility of α-, β-, and γ-cyclodextrins increases with increasing temperature. The solubility of β-cyclodextrin is lower than that of α- or γ-cyclodextrin. The solubility of β-cyclodextrin in water is only 0.8% at .5°C, rises to 1.35% at 15°C, to 1.55% at 20°C, and to 2.25% at 30°C. Cerestar repeats this disclosure in "Modified Cyclodextrins" which discloses in the bottom row of the table entitled "Properties of Cyclodextrins" that the solubility in water of betacyclodextrin at 25°C is 1.88%.

The solubility of BCD in water, as disclosed in the prior art, is summarized in the table below:

	5°C	15°C	20°C	25°C	30°C
Redenti, et al., Journal of Pharmaceutical Sciences, 89(1):1-8 (2000)	N/A	N/A	N/A	1.85%	N/A
"Cyclodextrins", Cerestar USA, Inc.	0.8%	1.35%	1.55%	N/A	2.25%
"Modified Cyclodextrins", by Cerestar Inc.	N/A	N/A	N/A	1.88%	N/A

Table - Published BCD Solubility in Water

Applicants submit, accordingly, that one skilled in the art would not be able to duplicate the disclosure of Kata, specifically that portion of the disclosure in which the

concentration of betacyclodextrin is at 2.25% and the temperature is below 30°C. Because it is known in the art that the aqueous solubility of betacyclodextrin at temperatures below 30°C is less than 2.25%, and is much lower at the refrigerator temperature of 6°C disclosed by Kata, such aqueous solutions containing 2.25% betacyclodextrin and 1.5% metronidazole are not obtainable. Applicants submit that the disclosure of Kata is not enabling for such solutions maintained at a temperature below 30°C, such as at room temperature or at 5°C.

This conclusion is in conformity with data obtained by the present inventors and disclosed in the present specification in Examples 1 and 2, at pages 11 and 12. As disclosed in Example 1, an aqueous gel composition containing various excipients and varying concentrations of betacyclodextrin was unstable at a temperature of 5°C when the concentration of betacyclodextrin was higher than 0.5% w/w.[1] Further, as disclosed in Example 2, such aqueous gel compositions containing the maximum obtainable dissolved concentration of betacyclodextrin were only capable of stably solubilizing 0.8 % w/w metronidazole.

As noted by the Examiner, Kata does not disclose the use of a combination of solubility enhancing agents. Chien discloses the use of solubility enhancing agents, such as the combination of N,N-dimethylacetamide, ethanol, and niacinamide, to increase the solubility of metronidazole in aqueous solution. Applicants respectfully submit that the present claims are patentable in view of the combined disclosure of Kata and Chien.

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[1] It is noted that the physically stable concentration of BCD in the aqueous formulation of Example 1 (0.5% at 5°C) is lower than that reported by Cerestar (0.8% at 0.5°C). This discrepancy is due to the fact that Cerestar disclosed solubility of BCD in water whereas Example 1 of the present specification discloses solubility of BCD in water that is also being used to dissolve additional compounds, including the gelling agent hydoxyethyl cellulose. As disclosed in Loftsson, U.S. Patent No. 5,324,718, at column 3, lines 57-60, "additives such as sodium chloride, buffer salts, surfactants and organic solvents (e.g. ethanol) usually reduce the solubilizing effects of cyclodextrins.

Preliminarily, Applicants herein repeat the arguments presented in the Amendment filed on February 2, 2004 which establish that the Examiner has failed to establish a prima facie case of obviousness in view of the combined disclosures of Kata and Chien. Secondarily, even if a prima facie case of obviousness is established by the prior art, Applicants submit that the present claims are patentable in view of the unexpected advantageous property of the combination of niacinamide (or niacin) and betacyclodextrin in the ability of this combination to dissolve metronidazole in aqueous solution compared to the ability of either niacinamide (or niacin) or betacyclodextrin alone.

The Examiner has reviewed the reasoning previously submitted by Applicants to establish synergy and has found such reasoning not to be persuasive. Applicants respectfully submit that the Examiner's reasoning is in error and submit that the data provided by Applicants does indeed establish the requisite synergy.

As reasoned by the Examiner, a solution of 0.5% BCD dissolves 8 units of MTZ and a solution of 3% niacinamide dissolves 10 units of MTZ, so that a solution of 1% niacinamide would be expected to dissolve 3.3 units of MTZ. Therefore, simply adding the dissolving properties of 0.5% BCD and 1% niacinamide would expectedly dissolve 11.3 units of MTZ. Therefore, the Examiner concludes that Applicants' showing of the dissolution of 10 units of MTZ by an aqueous solution containing 0.5% BCD and 1% niacinamide is not unexpected.

Applicants respectfully submit that the Examiner's reasoning might be correct if, and only if, BCD in water and niacinamide in water coexisted as separate solvent systems when the two agents (BCD and niacinamide) are combined in water. In such a case, one would expect that the solubility of a solute (ie MTZ) in the combined system would be the sum of the solubility

of the solute in each of the systems when not combined. Thus, MTZ would be expected to be dissolvable to a concentration that would equal the concentration obtained by adding BCD to water and the concentration obtained by adding niacinamide to water.

What the Examiner has not correctly considered is that there is a single solvent system and that the solvent is water and only water. BCD and niacinamide (or niacin) are not solvents but merely act to augment the solvent capacity of water. As clearly stated in the specification on page 3, lines 21-24, the terms "solubility enhancing agent" or "solubility enhancer" means a chemical compound that . . . increases the solubility of a second chemical compound . . . but which chemical compound is not itself a solvent for the second chemical compound." In the present case, water by itself[2] dissolves MTZ to a concentration of 0.7%. Water is not a solubility enhancing agent. BCD and niacinamide, solubility enhancing agents, do not dissolve MTZ, but rather increase the ability of an aqueous system to dissolve further amounts of MTZ than would be possible without the inclusion of these solubility enhancing agents.

In such a situation, the proper analysis to determine synergy is not to add the solubility of each of the separate systems, as the Examiner has done. Rather, the proper analysis is to determine if the additional solubility of MTZ obtained by combining BCD and niacinamide (or niacin) in aqueous fluid is higher than the additional solubility of MTZ obtained by using BCD in the fluid (without niacinamide or niacin) plus the additional solubility of MTZ obtained by using niacinamide (or niacin) in the fluid (without BCD). If so, then the combination of BCD and niacinamide (or niacin) has a synergistic activity on the aqueous solubility of MTZ.

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[2] Actually, the aqueous solution of Example 1 without BCD or niacinamide (or niacin).

In the present case, the solubility of MTZ in aqueous fluid without either BCD or niacinamide (or niacin) is 0.7 % w/w. This 0.7 % is the intrinsic solubility of MTZ in the fluid without the presence of a solubility enhancer.

The addition of 0.5% BCD to an aqueous fluid results in a MTZ solubility of 0.8%. This solubility is the 0.7 % which is the intrinsic solubility of MTZ in aqueous fluid plus 0.1% which is the enhancement of solubility in the system by the addition of the solubility enhancer, BCD.

The addition of 1% niacinamide to an aqueous fluid results in a MTZ solubility of 0.8 %. (See, Declaration of Yunik Chang cofiled herewith and discussed below.) This solubility includes the 0.7 % which is the intrinsic solubility of MTZ in aqueous fluid plus 0.1 % which is the enhancement of solubility in the system by the addition of the solubility enhancer, niacinamide.[3]

The sum of the solubility enhancement by 0.5% BCD and by 1% niacinamide is 0.2 % (0.1 % by the BCD and 0.1 % by the niacinamide). Thus, if the solubility enhancement of the combination of BCD and niacinamide were merely additive, one would expect a solubility enhancement of 0.2 %, or a total MTZ solubility of 0.9 % w/w.

However, as disclosed in the specification, in Example 3, Table 4, page 13, combining 0.5 % BCD and 1.0 % niacinamide resulted in a MTZ solubility of 1.0 % w/w, a total solubility enhancement of 0.3 %. This solubility enhancement is 50% higher than the sum of the

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[3] The enhanced solubility of 1% niacinamide may also be determined if one assumes linearity based on the data evaluated previously by the Examiner that 3% niacinamide results in a solubility of MTZ of 1.0 %. By subtracting the intrinsic aqueous solubility of MTZ of 0.7 %, the enhanced solubility of MTZ by 3% niacinamide is 0.3%. A 1 % niacinamide solution would be expected to enhance solubility of MTZ by one-third as much, or 0.1 %, which would result in a solubility of MTZ of 0.8 %.

solubility enhancements of the BCD and niacinamide alone. Because the solubility enhancement obtained by combining BCD and niacinamide is higher than that of the solubility enhancement of BCD alone plus the solubility enhancement of niacinamide alone, the data establish synergy of the BCD/niacinamide system. Similar results are shown for the combination of BCD and niacin.

The Examiner's analysis in the Office Action is erroneous as follows. The Examiner stated that 0.5% BCD will dissolve 8 units of MTZ. However, the Examiner did not consider that, of this 8 units of MTZ, 7 units were dissolved by the aqueous fluid even without BCD. The Examiner should not have considered the total solubility contribution of BCD to be 8 units, but rather 1 unit of solubility enhancement.

The Examiner further stated that 3% niacinamide will dissolve 10 units of MTZ. More correctly, water dissolves 7 units of MTZ and 3% niacinamide provides an additional MTZ solubility of 3 units. Assuming linearity as the Examiner did, a 1% niacinamide concentration would then be expected to solubilize, in addition to the intrinsic solubility due to water, 1 unit of MTZ.

Therefore, a solution containing 0.5 % BCD and 1.0 % niacinamide would be expected to provide a solubility enhancement of 2 units. This combined with the 7 units of inherent solubility of MTZ in water would yield an expected solubility of 9 units, not the 11 units determined erroneously by the Examiner. The Examiner's error was in neglecting to subtract the intrinsic solubility of MTZ in water before determining the solubility contribution of BCD and niacinamide.

The Examiner has also erred in the "apples-to-apples comparison" in the bridging paragraph of Pages 8 and 9 of the Office Action. The Examiner merely added the MTZ

solubility (0.8 %) obtained with 0.5 % BCD to the solubility obtained with the minimum amount of niacinamide required to prepare a 0.8 % MTZ solution. Data has shown that such amount of niacinamide is 1.0 %. The Examiner stated that, if the 0.5 % BCD and the niacinamide (1.0 %) combined would produce a greater than 1.6 % MTZ solution, this would show synergy.

Applicants agree that this would indeed show synergy, but disagree that such an incredibly high level of solubility (1.6 %) is needed to establish synergy. The Examiner's error is that the inherent solubility of MTZ in water has been included two times in the calculations. Of the 1.6 % theoretical solubility, 0.7 % is due to the inherent solubility of MTZ in water. This inherent solubility may be factored in, if desired, in the equation as follows:

> 0.7 % (inherent solubility in water) + 0.1 % (additional solubility due to BCD) + 0.1 % (additional solubility due to niacinamide) = 0.9 % (expected additive solubility due to BCD and niacinamide in water)

What the Examiner did, however, is as follows:

> 0.8 % (solubility due to 0.7 % inherent solubility in water + 0.1 % additional solubility due to BCD) + 0.8 % (solubility due to 0.7 % inherent solubility in water + 0.1 % additional solubility due to niacinamide) = 1.6 %.

In the Examiner's equation, the inherent solubility of water is added twice, not just once as it should have been. If one corrects this error by removing the second, erroneous contribution of water to the solubility of MTZ (0.7 %), the result is 0.9 %, which is the same as that expected by the analysis shown above by Applicants' method.

Accordingly, Applicants submit that the combination of BCD and niacinamide (or niacin) shows unexpected advantageous properties of synergy and is therefore not obvious in view of the combined disclosures of Kata and Chien.

In addition to the above, Applicants submit herewith a Declaration of Yunik Chang, one of the inventors of the present invention. In his Declaration, Yunik Chang presents data to establish the synergistic effect obtained by combining betacyclodextrin and niacinamide. Data in the Declaration shows (1) that combining 1% niacinamide and 0.5% betacyclodextrin results in solubility enhancement of metronidazole in aqueous fluid that is greater than would be expected by adding the solubility enhancement qualities of the niacinamide and the betacyclodextrin and (2) that niacinamide provides for increased solubility of betacyclodextrin, which further results in increased solubility of metronidazole. This last finding is conjectured by Yunik Chang to be a reason why niacinamide and betacyclodextrin function synergistically when combined together.

In view of the above, Applicants submit that the synergistic activity of the combination of betacyclodextrin and niacinamide has been unequivocally established and the Examiner is requested to reconsider and to withdraw the rejection of these claims on the basis of obviousness.

B. Kata, Acta Pharm. Hung, 54:116-122 (1984), Chien, U.S. Patent No. 4,032,645, and Czernielsewski, U.S. Patent No. 5,849,776

The Examiner has rejected claims 24, 26-68, 70-74, and 76-90 as being obvious over Kata and Chien in view of Czernielsewski, U.S. Patent No. 5,849,776. Applicants traverse the rejection of these claims on this ground.

The disclosure of Czernielsewski is cited for its disclosure of an aqueous gel composition comprising up to 5% metronidazole by weight. The Examiner further stated that it would be obvious to prepare a gel having an MTZ concentration higher than 1% using the solubilizing agents disclosed by Kata and Chien.

Applicants submit that the disclosure of Czernielsewski is not enabling for a solution containing a dissolved concentration of metronidazole higher than 0.75 %. The problem of obtaining an aqueous concentration of metronidazole higher than 0.75 % has confronted the art for a very long time. Czernielsewski stated that the aqueous compositions may contain metronidazole at a concentration of up to 5 % (column 2, lines 42-48). However, Czernielsewski does not disclose whether such compositions are suspension gel compositions or solution gel compositions. Czernielsewski does not disclose how a solution composition having a MTZ concentration higher than 0.75 % may be made. Additionally, each of the examples of Czernielsewski illustrate the invention with a MTZ concentration of 0.75 %. One skilled in the art may not make a solution composition of MTZ higher than 0.75 % by following the disclosure of Czernielsewski, except by further invention, that is by applying an undue level of experimentation. Although the disclosure of Czernielsewski may or may not be enabling for the production of suspension compositions having a concentration of metronidazole higher than

0.75%, the disclosure of Czernielsewski does not enable the production of aqueous solution compositions having a concentration of MTZ higher than 0.75%.

In the present application, Applicants have provided such further invention by determining that a concentration of MTZ higher than 0.75 % may be obtained by combining BCD and niacinamide or niacin in water. Applicants submit, therefore, that combining the Czernielsewski reference with the disclosures of Kata and Chien does not provide a prima facie case of obviousness for rejecting these claims.

In the event that the Examiner does not agree with this analysis and persists in holding that the combination of Kata, Chien, and Czernielsewski provides a basis for prima facie obviousness of these claims, Applicants submit that the unexpected advantageous property of synergy as discussed above supports the patentability of these claims over this combination of references.

For these reasons, Applicants submit that claims 24, 26-68, 70-74, and 76-90 are not obviousness over the combined disclosure of Kata, Chien, and Czernielsewski.

In addition to the above, Applicants note that the Examiner has included a rejection of claims 89 and 90 as being obvious in view of Kata, Chien, and Czernielsewski. Applicants submit that, in addition to the reasons for patentability discussed above, these claims are patentable over these references for the following reasons.

The invention claimed in these claims is not pertinent in any way to the disclosure of Czernielsewski. These claims call for a method for increasing the solubility of BCD in water by combining with niacinamide. Czernielsewski discloses neither of these two compounds.

Neither Kata nor Chien discloses that the solubility of a solubility enhancer itself may be increased by adding a second solubility enhancer.

The present specification discloses that the maximum soluble concentration of BCD in aqueous solution is 0.5 %. See Example 1, Table 2, pages 11-12. However, when combined with niacinamide, a stable dissolved concentration of 1.0 % BCD is obtained. See Example 3, Table 4, page 13 of the specification.

This result, the increased solubility of the solubility enhancer BCD by adding niacinamide to an aqueous solution, is unexpected and advantageous. Applicants submit that this provides an additional and compelling rationale for the patentability of claims 89, and claims dependent therefrom.

For these reasons, Applicants respectfully request that the Examiner reconsider and withdraw the rejection of claims 24, 26-68, 70-74, and 76-90 on this ground.

C. Kata, Acta Pharm. Hung, 54:116-122 (1984), Chien, U.S. Patent No. 4,032,645, and Loftsson, U.S. Patent No. 5,324,718

The Examiner has rejected claims 24, 25, 27-29, 31, 32, 40, 41, 43-58, 63, 65-69, 71-87, 89, and 90 as being obvious in view of the combined disclosure of Kata, Chien, and Loftsson, U.S. Patent No. 5,324,718. Applicants traverse the rejections of these claims on this ground.

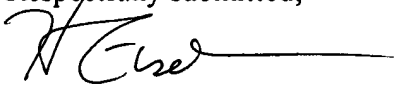
The Examiner has cited the Loftsson reference in combination with the Kata and Chien references for its disclosure of adding MTZ to a solution after the other agents are dissolved in water.

Applicants submit that these claims are patentable for the reasons submitted above pertaining to rejection of claims for obviousness based on the combination of Kata and Chien or the combination of Kata, Chien, and Czernielsewski. Accordingly, the Examiner is requested to reconsider and to withdraw the rejection of claims 24, 25, 27-29, 31, 32, 40, 41, 43-58, 63, 65-69, 71-87, 89, and 90 on this ground.

CONCLUSION

Applicants submit that the claims are in condition for allowance. The Examiner is requested to withdraw all present grounds for rejection of the claims and to promptly issue a notice of allowance.

Respectfully submitted,



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Enclosures: Declaration of Yunik Chang
Redenti, et al., Journal of Pharmaceutical Sciences, 89(1):1-8 (2000),
"Cyclodextrins", Cerestar USA, Inc., and
"Modified Cyclodextrins", by Cerestar Inc.

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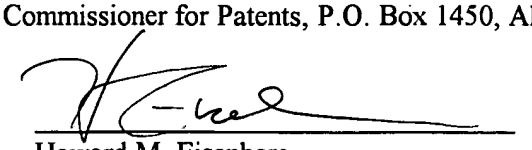

Howard M. Eisenberg

MINI-REVIEW

Drug/Cyclodextrin/Hydroxy Acid Multicomponent Systems. Properties and Pharmaceutical Applications

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ABSTRACT: The objective of this mini-review is to summarize the findings concerning the properties and the pharmaceutical applications of multicomponent complexes made of a sparingly water-soluble amino-type drug, a cyclodextrin, and a hydroxy carboxylic acid. Simultaneous complexation and salt formation with these acids significantly increase the solubilizing power, allowing us to reduce the amount of cyclodextrin necessary for making the targeted formulation. In many cases, the aqueous solubility of the hydrophobic drug can be enhanced by several orders of magnitude, while that of CD can be enhanced more than 10-fold. The mechanism through which these complexes elicit their synergetic effects on the drug solubility is also discussed. Finally, some general observations are made concerning the structural requirements of the drug necessary for exploiting the aforementioned effect. © 2000 Wiley-Liss, Inc. and the American Pharmaceutical Association J Pharm Sci 89: 1–8, 2000

INTRODUCTION

Cyclodextrins (CDs) are a family of cyclic oligosaccharides, the most common α, β, and γ, consisting of 6–8 D-glucopyranosyl units, linked by α-(1→4)-glycosidic bonds. They have a toroidal shape with all secondary hydroxyl groups, O2-H and O(3)-H, located on the wider rim and all primary hydroxyl groups, O(6)-H, on the narrower rim. The glucose units are in the $^4C_1$ chair conformation. The hydrogen atoms and glycosidic oxygen atoms are located on the inner surface of the cavity, which is relatively hydrophobic. Intramolecular hydrogen bonds O(3)-H · · · O(2)-H or O(3) · · · H-O(2) exist between the secondary hydroxyl groups of adjacent glucose units.[1,2]

By virtue of their shape, CDs can accommodate a variety of molecules inside the cavity to form inclusion compounds. The guest molecules, in turn, surrounded or encapsulated by CDs, may experience some changes in their physical, chemical, or biological properties. In the pharmaceutical industry, this feature has been advantageously exploited for increasing the stability and bioavailability of drugs, reducing irritation and other side effects, and improving palatability and handling. For further information on their application, the reader is referred to several excellent books and monographs published in recent years.[3–7]

One of the most important application of CDs still remains the possibility of increasing the aqueous solubility of sparingly soluble drugs[8,9]

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especially in alternative to other techniques such as the use of co-solvents or surfactants and lipid emulsion formation.[10] However, natural CDs are generally not very soluble in water because of the relatively strong binding of the molecules in the crystal state (i.e., relatively high crystal lattice energy). In particular β-cyclodextrin (βCD), the most widely used member of the family by virtue of its cavity size, availability, and low cost, exhibits limited aqueous solubility (1.85% at 25°C), which often hinders its successful application as solubilizing agent. For instance, βCD is about 7-fold less soluble than αCD and 14-fold less than γCD. The most probable explanation is based on the fact that the arrangement of its secondary hydroxyl groups is optimal for intramolecular hydrogen bond interaction, preventing adequate hydration by water molecules.[6,11] An alternative explanation of the abnormally low solubility of βCD was proposed by Coleman and co-workers who invoked the formation of aggregates and their unfavorable interaction with the hydrogen-bonded structure of water;[12] however, another research group, on the basis of T_1 relaxation measurements, raised well-grounded concerns on the existence of aggregates.[13] Moreover, the addition of βCD to the aqueous drug solutions or suspensions often results in the precipitation of the corresponding CD complexes as it gives rise to a B_s type phase solubility diagram.[14]

For several reasons, including toxicology, cost, and dosage, the amount of cyclodextrin to be used in most drug formulation should be limited. Therefore, different approaches have been undertaken for enhancing the solubilizing power of parent CDs:

(i) chemical substitution of any of the hydrogen bond forming hydroxyl groups; the research in this area has been particularly intensive and many derivatives that are more soluble in water are reported in the literature;[15–18]

(ii) addition of water-soluble polymers such as polyvinylpyrrolidone or hydroxypropylmethycellulose with the aim of increasing the apparent stability constant;[19–21]

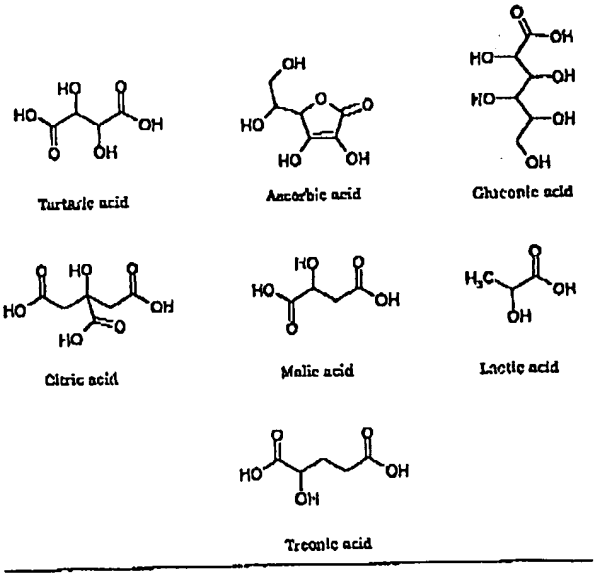
(iii) combination of appropriate pH adjustment and complexation; although CD complexes with un-ionized drugs are usually more stable than with their ionic counterparts,[22,23] nevertheless the achieved total solubility (free ionized drug + free un-ionized drug + ionized drug complex + un-ionized drug complex) can be significantly increased.[24–31]

The present paper is intended to give a survey on the properties and pharmaceutical applications of amino-type drug–hydroxycarboxylic acid–cyclodextrin multicomponent complexes: The addition of hydroxy carboxylic acids (HAs), such as citric, malic, lactic, or tartaric (Table I) yields freely water-soluble complexes that can be isolated by freeze-drying or spray-drying. The resulting amorphous compounds dissolve very rapidly and give rise to supersaturated solutions that remain stable for several days. The synergetic mutual enhancement of both drug and cyclodextrin aqueous concentration,[32,33] the latter being extremely evident when members with low solubility are used, such as βCD, relies partially on the specific interaction of the hydroxy acid groups with the hydrogen bond system of the host and/or the modification of the hydrogen bond network of the surrounding water molecules.

PROPERTIES AND PHARMACEUTICAL APPLICATIONS

Many papers in the literature deal with the outstanding solubility properties of complexes prepared in presence of hydroxy acids. Advantages in terms of bioavailability and pharmaceutical use are often described or convincingly envisaged.

TABLE I. Structure of the Examined Hydroxy Carboxylic Acids



The aqueous solubility of terfenadine (TFN), a selective histamine H_1 receptor antagonist with no adverse sedative effect, was dramatically increased by simultaneous βCD complex formation and salt formation with tartaric, citric, or ascorbic acid. The TFN concentration after dissolution of the relevant complexes turned out to be 30–50 mg/mL, corresponding to 3,000–5,000-fold solubility enhancement with respect to the drug itself and to 400–600-fold with respect to the binary complex. The dissolved βCD concentration increases up to 160–200 mg/mL, which means about 10-fold solubility enhancements.[34] This approach allows us to significantly reduce the amount of CD necessary for the solubilization of the same amount of drug as well as the volume of water necessary for freeze- or spray-drying to obtain the solid complexes.[33,34] An oral bioavailability study was carried out on 8 fasted rabbits in a cross-over design. The animal received 10 mg/kg of TFN as 1:2:1 βCD multicomponent complex with tartaric acid and as suspension. Blood samples were drawn for approximately 24 h postdosing, and TNF carboxylate metabolite levels were determined by HPLC.[35] The multicomponent complex gave a better absorption profile in terms of C_{max} and T_{max}, but the bioavailability of TFN was not significantly affected, probably due to the large stability constant of the complex.[36,37]

Besides oral administration, the complexes seem to be more suitable for nasal spray formulation in rhinitis treatment.

Similar or even better solubility enhancement results were achieved with other structurally related derivatives (i.e., amino-type drugs bearing a diphenylmethane moiety) such as cinnarizine, a cerebral vasodilating agent,[32] and tamoxifen and clomiphen, two well-known anti-estrogens.[38]

In the case of βCD multicomponent complexes of *cis*-ketoconazole (KC), an imidazole antifungal agent, with tartaric or citric acid, 2,200- and 80-fold KC solubility enhancements were achieved in comparison to the drug itself and to the binary complex, respectively.[32,39] Following oral administration in fasted rabbits at 15 mg/kg, the bioavailability (AUC_{0-6h}) of KC was 1.4-fold higher than the reference formulation (0.5% methylcellulose suspension).[39] The acidic microenvironment created by the complex facilitates the dissolution of KC at higher pH values (5–6) as well;[40–42] a bioavailability improvement may hence be envisaged in patients with increased gastric pH, a condition associated with some diseases such as AIDS and cancer. In a comparative three-way study in rabbits with elevated gastric pH for omeoprazole, multicomponent formation increases KC absorption 6 times over that of the binary complex; a suspension of KC was practically not absorbed.[42] An in vitro–in vivo (IVIV) correlation between dissolution/solubility and absorption was subsequently demonstrated.[43]

Other drugs belonging to different chemical classes (benzimidazole and tricyclic derivatives) are suitable for forming multicomponent complexes with hydroxy acids. Astemizole (AST), another potent and long-acting histamine H_1 receptor antagonist, upon βCD muticomponent complexation, gives stable solutions with 85–188 mg/mL AST concentration corresponding to 17,000–27,600-fold enhancement of solubility. Curiously, the enantiomeric forms of tartaric acid were found to be more effective than the racemic mixture.[44] Cyclobenzaprine (CBP), which is indicated in the relief of muscle spasms, forms freely soluble and stable βCD multicomponent complexes in spite of the unusually high βCD concentration (about 15% w/v). The remarkable improvement of CBP solubility (about 780-fold) could allow us to reduce the dose as well as the concomitant side effects.[45]

In addition to βCD, hydroxy acids also turned out to be effective in intensifying the solubilization power of α- and γ-CD[32,33] as well as of some CD derivatives such as hydoxypropyl-β-cyclodextrin (HPβCD) and sulfobutylether-β-cyclodextrin (SBE-βCD).

Mura *et al.*,[46] in a study investigating the effects of different acids and cyclodextrins on the aqueous solubility of econazole, an imidazole antifungal agent, found that the best results could be achieved by the simultaneous use of α-CD and lactic acid in a 1:2.5 molar ratio.

Fenyvesi *et al.*[47] found that much less HPβCD could be used for dissolving the therapeutical doses of terfenadine, astemizole, and domperidone by using hydroxy acids. The necessary amounts were respectively reduced to 1/15, 1/100, and 1/350, in comparison to the binary complexes, without use of co-solvents such as ethanol.[48,49]

Piel *et al.*[50] developed non-surfactant parenteral formulations of miconazole (MIC), an imidazole antifungal drug, by exploiting the synergistic solubilization effect between lactic acid or gluconic acid and HPβCD or SBE_7-βCD. The formulations with lactic acid were compared to a marketed micellar solution containing castor oil after intravenous administration to six sheep. The pharmacokinetics of MIC were identical,[51] indi-

cating that this approach can be proposed as a good alternative to the use of surfactants. More recently, the same research group provided additional data showing the usefulness of the same approach in the parenteral administration of albendazole, an antihelminthic drug.[52]

PHYSICOCHEMICAL CHARACTERIZATION AND STRUCTURAL INVESTIGATION

The term "multicomponent" has been previously used for describing either CDs complexes of mixture of different substances,[53,54] or self-organized assemblies with amphiphilic compounds (including amphiphilic CDs).[55,56] It was extended by us to the systems herein reviewed, as it was originally hypothesized that the potential surfactant properties of the salts of the drugs included and their supposed ability of forming micelle even in presence of CDs[57,58] might be responsible for the observed synergetic solubility enhancement. In a preliminary NMR study carried out on the TFN/β-CD/HA (tartaric and citric acids) systems, it was indeed found that the relaxation time and hence the mobility of the counter-ion was considerably reduced in solution, suggesting the possible presence of aggregates.[59] However, a further study by dynamic light scattering on the same systems ruled out these conclusions as only particles with a mean diameter of 1.4–2.5 nm (i.e., monomers or at the most dimers) could be detected.[60] This finding agrees with what has been reported by Bellanger *et al.*[13] NMR data, particularly NOE measurements, also helped us to exclude that the synergetic solubilizing effect was due to the simultaneous accommodation of the hydroxy acid and the drug into the CD cavity, as observed for other ternary systems.[61,62] Although the employed hydroxy acids can form complexes with a well-measurable association constant,[63–65] nevertheless, they are displaced from the cavity by the stronger binding of the aromatic moieties of TFN, in particular the *p-tert*-butylphenyl ring.[66,67] In a more recent study, aiming at investigating the interaction of the enantiomers of *cis*-KC with βCD in the presence of (+)-tartaric acid, T_1 relaxation measurements indicated that the mobility of the counter-ion was significantly reduced even in this case. A theoretical model built up on the basis on the experimental NMR data, besides confirming that inclusion occurs form the wider-diameter side of βCD by accommodation of the dichlorophenyl ring, also suggests that tartrate ion is probably strictly involved in the molecular assembly by establishing electrostatic interaction with the imidazolium ring of KC and hydrogen bonds with the hydroxyl groups of βCD.[68] Indirect evidence of such outer-sphere interaction derived from ion spray mass spectrometry experiments performed on the same TFN and KC complexes. In both cases, a 1:1:1 drug–CD–HA adduct was observed[69,70] whose survival in the gas phase could be explained only supposing both non-covalent binding with the hydrophobic moiety and polar-type interaction between hydrophilic tartrate anion and βCD. The X-ray structure determination of the single crystal of 1:2:0.5 TFN/βCD/(+)TA complex, currently in progress, will be able to cast more light on the interaction among the partners.[71]

The capability of high concentrations of hydroxy acids, in particular citric acid, of enhancing the aqueous solubility of βCD has been observed by different research groups.[64,65] It was also suggested that the effect relies on the capability of the hydroxy acids to modify the intramolecular hydrogen bond system involving the secondary hydroxyl groups of CDs and/or affect their interaction with the surrounding water molecules.

These findings may help explain the results achieved via multicomponent technology. In fact, the solubility of the corresponding complexes is much higher than that which could be expected by increasing the total drug solubility through only appropriate pH adjustment. The drugs that elicit the most outstanding solubility improvement are strongly bound to the CD cavity even though they are in the ionized form. Their basic center and hence the charge are indeed located quite far away from the part of the molecule included, so the complexing ability is not significantly compromised. The hydroxy acid is kept in the proximity of the external rim of CD by a concerted mechanism that involves the binding of the hydrophobic part of the drug and simultaneous formation of a strong ion pair. The latter, i.e., that dissociation of ion pair is hindered by inclusion complexation, has also been suggested by other authors,[72] who explained the observed differential solubility of different ziprasidone salt forms in SBE-CD solution by invoking a difference in the degree of ion-pair formation. So, the stronger the ion pair, the more effective the capability of the hydroxy acid of interacting with the external hydrogen bond system of CDs. Accordingly, a smaller amount is needed to exert its solubiliza-

tion effect on the host and, in turn, on the complexed drug.

The experimental and theoretical observations and some partially negative results obtained[73–75] allow us to indicate a number of requirements for exploiting the properties of the multicompenent complexation technology: (i) drug solubility should be low (less than 0.1 mg/mL) and should be improved by salt formation with hydroxy acids; (ii) a relatively tight fit between the complexed part of the molecule and the CD must occur (K_{ass} at least 10^3 M^{-1}); (iii) the amino group should have a fairly basic character ($pK_a \geq 5.0$) and must be located far away from the part the molecule included in such a way as that either the charge does not compromise the complexing ability and the counter-ion is able to interact with the hydroxyl groups on the wider external rim of CD.

CONCLUSIONS

Although the use of cyclodextrins as pharmaceutical excipients has been rapidly increased and promoted, and new safer derivatives are constantly being developed, nevertheless, for several reasons, including toxicology, cost, and dosage, the amount of cyclodextrin to be used in most drug formulation should be limited.

Multicomponent complex formation in the presence of hydroxy acids is a useful and powerful approach for improving the solubilizing power of cyclodextrins and therefore reducing their necessary amount. The reported examples show that the solubility of the hydrophobic drug can be enhanced by several orders of magnitude, while that of CD can be enhanced more than 10-fold. Oral formulations with increased dissolution rate and bioavailability of the complexed drug over a wider pH range can be prepared accordingly. Alternatively, a stable solution for local or parenteral administration can be generated without using organic cosolvents, surfactants, or lipids by employing a reduced amount of HPβCD or SBE-βCD.

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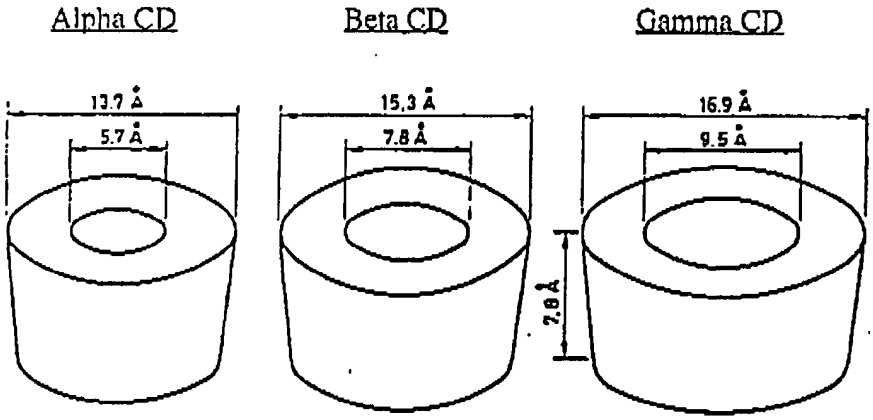
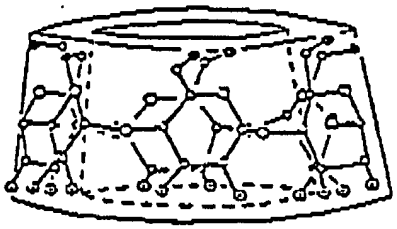
Cyclodextrins are produced by a highly selective enzymatic synthesis. They consist of six, seven, or eight glucose monomers arranged in a donut shaped ring, which are denoted alpha, beta or gamma cyclodextrin respectively.

The specific coupling of the glucose monomers gives the cyclodextrins a rigid, conical molecular structure with a hollow interior of a specific volume.

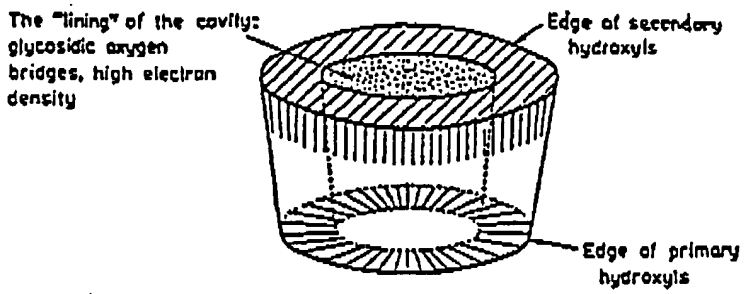


Dimensions of cyclodextrins

This internal cavity, which is hydrophobic in its nature, is a key structural feature of the cyclodextrins, providing the ability to complex and contain a variety of "guest" molecules (e.g., aromatics, alcohols, halides and hydrogen halides, fatty acids and their esters, etc.). The guests must satisfy the size criterion of fitting at least partially into the cyclodextrin internal cavity, resulting in an inclusion complex.



Chemical Structure of α-CD

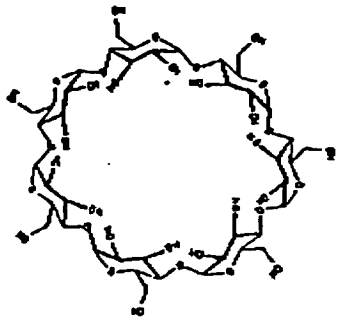


Functional structural scheme of cyclodextrins

It is this unique ability of cyclodextrins to molecularly encapsulate and thereby modify the apparent physical and chemical properties of guest molecules that is the object of much current chemical research.

Learning how to take advantage of the behavior of cyclodextrin inclusion complexes to bring about improvements such as stabilization of light or oxygen - sensitive materials, control of biologically active substances (e.g., pharmaceuticals, unsaturated or volatile essential oils, etc.), and the modification of the chemical activity of guest molecules are all objects of Cerestar USA's cyclodextrin technology development. This natural biopolymer offers a new dimension to concepts of encapsulation and controlled release.

PROPERTIES CD	α-CD	β-CD	γ-CD
Degree of Polymerization	6	7	8
Molecular size /A°			
inside diameter	~ 5.7	~ 7.8	~ 9.5
outside diameter	~ 13.7	~ 15.3	~ 16.9
height	~ 7.0	~ 7.0	~ 7.0
Specific Rotation $[\alpha]_D^{25}$	+150.5	+162.5	+177.4
Color of Iodine complex	Blue	Yellow	Yellowish Brown
Solubility in water (g/100 ml) 25° C			
Distilled Water	14.50	1.85	23.20
Saturated solution of guest compounds,			
Guest Compounds:			
Cyclohexane	0.22	0.01	0.45
Bromobenzene	2.45	0.04	0.01
Fluorobenzene	1.08	0.06	0.03
p-cymene	2.74	0.06	0.17
Diethyl ether	3.80	0.61	0.28



The chemical structure of β-cyclodextrin.

Solubility in Water

(g./100 ml)

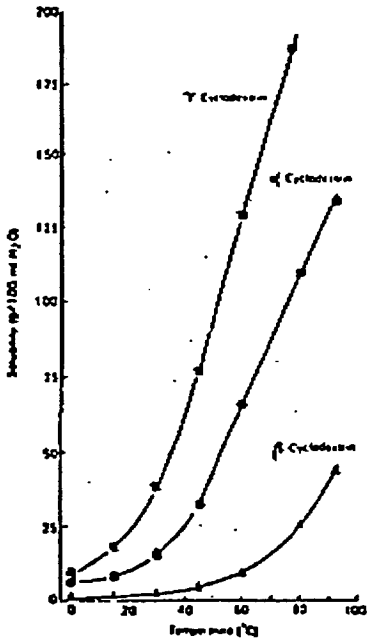
Temp. (°C)	α-CD	β-CD	γ-CD
0.5	6.8	0.80	9.1
15.0	8.5	1.35	18.4
20.0	10.1	1.55	23.2
25.0	13.0	1.85	30.0
30.0	16.0	2.25	38.5
40.0	25.6	3.52	63.5
45.0	32.3	4.45	77.2
50.0	49.5	5.62	93.8
60.0	66.2	9.02	129.2
70.0	87.6	15.30	163.7
80.0	109.3	25.30	198.0
90.0	131.6	39.70	—

Solubility in Selected Solvents

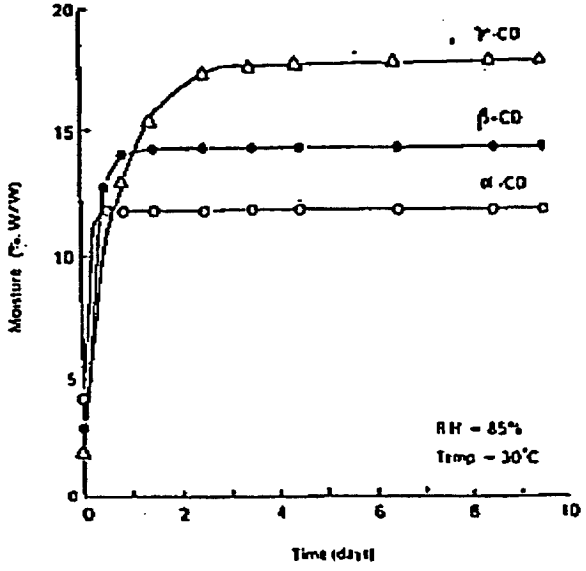
(%, w/v)

At 25°C Water:Solvent	α-CD 50/50	α-CD 0/100	β-CD 50/50	β-CD 0/100	γ-CD 50/50	γ-CD 0/100
Methanol	1.2	<0.1	0.3	<0.1	2.8	<0.1
Ethanol	0.9	<0.1	1.3	<0.1	2.1	<0.1
Propanol	0.8	<0.1	1.1	<0.1	0.7	<0.1
Isopropanol	4.7	<0.1	2.6	<0.1	0.6	<0.1
Acetone	1.9	<0.1	0.3	<0.1	0.5	<0.1

Water solubility of cyclodextrins



Hygroscopicity of α-, β- and γ-cyclodextrins



Modified Cyclodextrins

Fundamental knowledge of cyclodextrin inclusion phenomena, derived from over a decade of active international research, is now being translated into specialized performance oriented applications across a broad spectrum of industries, e.g. agro-chemicals, chemical processing, personal care, foodstuffs, and pharmaceuticals.

The initial promise of these innovations has in turn stimulated global research efforts designed to open up new application strategies through derivatization of the cyclodextrins. These lines of scientific inquiry have focused on the attachment of functionalizing groups to the parent cyclodextrin to obtain enhanced specific adduct binding for selective separations, purifications, and CD HOST-GUEST delivery systems, or to improve cyclodextrin catalytic properties in the design of synthetic enzymes.

In support of these explorations, Cerestar is currently making available the following developmental products for application research activity:

(A) Randomly methylated cyclodextrin

(B) Hydroxyethyl and hydroxypropyl cyclodextrin

(C) Sulfated cyclodextrin

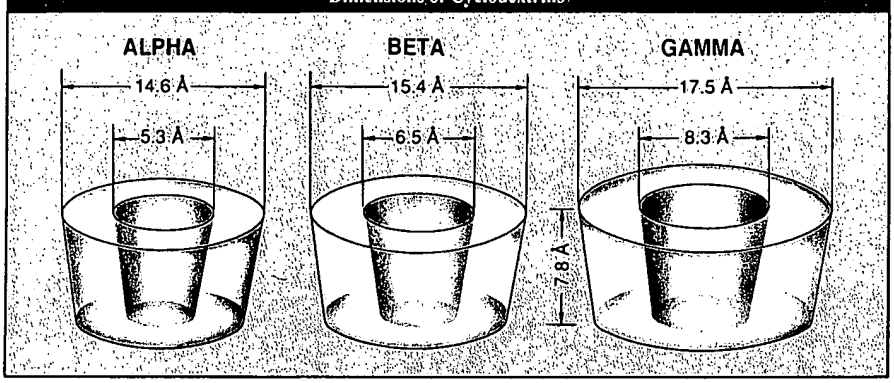
(D) Cyclodextrin polymer - (insoluble resin bead forms)

Cerestar has committed to this innovative technology. And we're bringing it to you, today, with North America's first commercial plant and first Cyclodextrin Technology Center.

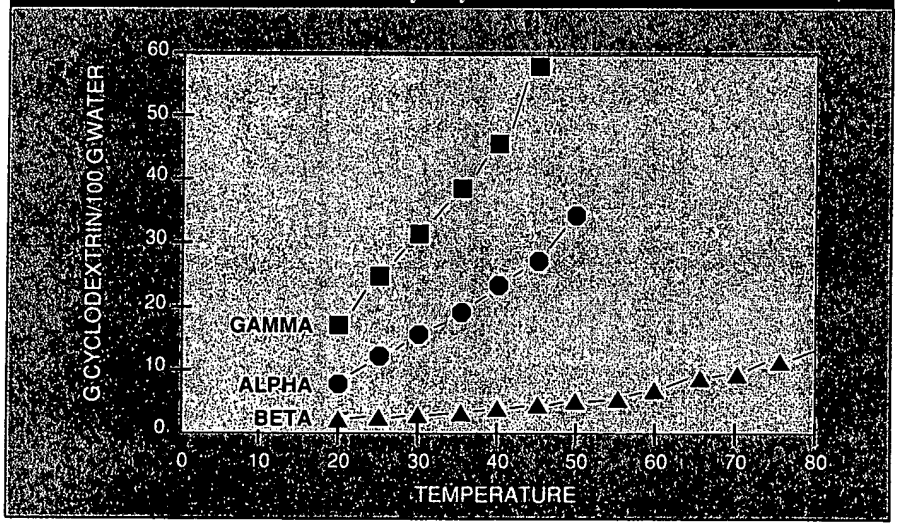
Further Information on complexation, assay techniques, and regulatory status is available upon request.

The future in cyclodextrins is here and now. So, take the lead and call us today...or follow the competitor who did.

Dimensions of Cyclodextrins



Solubility of Cyclodextrins



Properties of Cyclodextrins

Properties	Alpha-CD	Beta-CD	Gamma-CD
Glucose Units	6	7	8
Molecular Weight	973	1135	1297
Cavity Dimensions			
Cavity Diameter (Å)	4.7-5.3	6.0-6.5	7.5-8.3
Cavity Depth (Å)	7.9	7.9	7.9
Cavity Volume (Å)	174	262	472
Specific Rotation [α]$_D^{25}$	+150.5	+162.5	+174.4
Solubility in Water (g/100ml) 25°C	12.7	1.88	25.6

Solubility in Selected Solvents (%. w/v)

At 25°C	α-CD		β-CD		γ-CD	
Water/Solvent	50/50	0/100	50/50	0/100	50/50	0/100
Methonal	1.2	<0.1	0.3	<0.1	22.8	<0.1
Ethanol	0.9	<0.1	1.3	<0.1	2.1	<0.1
Propanol	0.8	<0.1	1.1	<0.1	0.7	<0.1
Isopropanol	4.7	<0.1	2.6	<0.1	0.6	<0.1
Acetone	1.9	<0.1	0.3	<0.1	0.5	<0.1

Beta Cyclodextrin Solubilities in Organic Solvents

Determined by Refractive Index at 25°C	
Solvent	Solubility (Per Cent wt/wt)
Ethylene Glycol	7.0
Propylene Glycol	1-0.5
Dimethyl Sulfoxide	1-41
Dimethyl Formamide	28.3
N-Methyl Pyrrolidone	14.8
Tetrahydrofuran	Nil
Methyl Isobutyl Ketone	Nil
Methyl Isopropyl Ketone	Nil
Acetone	Nil

1- 41% is highest level attempted - solution is very viscous